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Effect of nutritional supplement based on melatonin on the intraocular pressure in normotensive subjects

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Abstract:	Purpose: To evaluate the effect of a new nutritional supplement based on melatonin on the intraocular pressure (IOP) in normotensive subjects. Patients and Methods: A short-term and prospective study was designed. Sixty-seven normotensive subjects were recruited. Patients were divided in two groups. The daily group (DG) (n=18) were instructed to take the supplement from 22.00 to 23.00 (before sleeping) during 3 consecutive days. IOP was measured from 10.00 to 11.00 am the day before treatment and during the 3 days of experiment. The acute group (AG) (n=49) was instructed to take the supplement after the second measure (11.00) of the second day. IOP was measured 1 hour and just before the intake of the supplement and one and two hours after. All measures in this group were taken one day before without any supplement (control) and the day of experiment. Results: The DG group showed a significant decreasing in IOP after supplement intake in all days of experiment, from 14.9±3.4 mmHg to 13.8±2.9 mmHg after 3 days of experiment (p value<0.001). For AG, IOP did not change during the control day, however, a reduction of 1 mmHg was found two and three hours after supplement intake, from 15.7±2.5 mmHg to 14.7±2.5 mmHg and 15.1±2.7 mmHg, respectively, being statistically significant (p value<0.001). Conclusion: The supplement based on melatonin was able to reduce the IOP in normotensives subjects after 2 hours of intake. Moreover, the daily intake showed a reduction of IOP during the three days of experiment.
Response to Reviewers:	Reviewer #1: The work is an interesting one, which offers another target for the treatment of ocular hypertension and glaucoma, but the methodology is quite worrisome.

	The findings from this study would have been objective, if the study were properly designed. The study design would have been a controlled randomised clinical trial, to elucidate the objective effect of melatonin on IOP. The purpose of this study was to corroborate the effect of melatonin on IOP of normotensive patients that was achieve many years ago by Samples (Effect of melatonin on intraocular pressure, Samples JR, Krause G, Lewy AJ. Curr Eye Res. 1988 Jul;7(7):649-53), and to probe that melatonin not only decrease IOP in an acute way, but also that this effect could be detected after the acute effect finished. Although there is no placebo group in this study, there is a basal IOP from all patients at the same time points studied, that has a better correlation with the final IOP than comparing two different groups. It would have been age and sex-matched, with equal number of participants in each arm, who were not statistically different. While there is a difference between male and female patients (1.8:1 ratio), there is no correlation between gender and IOP excluding pregnancy and breastfeeding (Gender difference in the pathophysiology and treatment of glaucoma. Tehrani S Curr Eye Res. 2015 Feb;40(2):191-200) and there is no report about this possibility in melatonin's effect. There should have been a control group, who were given placebo. Although there is no placebo group in this study, there is a basal IOP from all patients at the same time points studied, that has a better correlation with the final IOP than comparing two different groups. That is why we decided to make crossed study better than a parallel one with placebo group. Considering the diurnal / nocturnal variations in IOP, the exact time of ingestion of the supplement for both groups should have been specified. All patients from AG received the supplement from 10:00 to 11:00 with measures the day before and the same day. Patients from DG received the supplement at 22:00 the 3 days. Both sentences has been added to the text in abstract and at line 68, in order to clarify that. IOP values from the placebo group would have ruled out the diurnal / nocturnal variations and highlighted the significance of the values in DG and AG. We know that there is a variation in IOP during all day, and that is why we have decided to take the intraocular pressure at the same time during both days (the control one and the treated one). With these two days, we are able to compare which is the normal diurnal variation and which is made by melatonin supplement. On the DG, there is also measures from the first day without any treatment, which can be also considered as basal values. In order to compare the results, the two first measures of the first day (10:00 and 11:00) were used as basal values and the same measures were made on the others to avoid day variations. Why was it only one-time ingestion by AG and not for 3 mornings? Because the main outcome of this group was to assess its acute effect which last from 2 to 4 hours, not the daily effect. Taking into account that the same patients could not be used for both experiments, we decided to split them and check separately the acute effect and the daily or chronic treatment. Why were the participants not examined to confirm the absence of normal pressure glaucoma? Patients were examined and consulted about this point on its anamnesis to ensure there were no ophthalmic diseases like glaucoma or any retinal one. We have included in the text at line 54.

Effect of nutritional supplement based on melatonin on the intraocular pressure in normotensive subjects

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Conflict of interest: Gonzalo Carracedo declares that he has no conflict of interest; Alejandro Martinez-Aguila that he is employee of Avizor SA; Candela Rodriguez-Pomar declares that she is employee of Avizor SA; Julia Bodas declares that she has no conflict of interest; Juan Sanchez declares that he has no conflict of interest; Jesus Pintor declares that he has no conflict of interest

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ABSTRACT

Purpose: To evaluate the effect of a new nutritional supplement based on melatonin on the intraocular pressure (IOP) in normotensive subjects.

Patients and Methods: A short-term and prospective study was designed. Sixty-seven normotensive subjects were recruited. Patients were divided in two groups. The daily group (DG) (n=18) were instructed to take the supplement ~~at night~~ from 22.00 to 23.00 (before sleeping) during 3 consecutive days. IOP was measured ~~at~~ from 10.00 to 11.00 am the day before treatment and during the 3 days of experiment. The acute group (AG) (n=49) was instructed to take the supplement after the second measure (11.00) of the second day~~in the morning only one day~~. IOP was measured 1 hour and just before the intake of the supplement and one and two hours after. All measures in this group were taken one day ~~after~~ before without any supplement (control) and the day of experiment.

~~-~~**Results:** The DG group showed a significant decreasing in IOP after supplement intake in all days of experiment, from 14.9±3.4 mmHg to 13.8±2.9 mmHg after 3 days of experiment (p value<0.001). For AG, IOP did not change during the control day, however, a reduction of 1 mmHg was found two and three hours after supplement intake, from 15.7±2.5 mmHg to 14.7±2.5 mmHg and 15.1±2.7 mmHg, respectively, being statistically significant (p value<0.001).

Conclusion: The supplement based on melatonin was able to reduce the IOP in normotensives subjects after 2 hours of intake. Moreover, the daily intake showed a reduction of IOP during ~~all~~ the three days of experiment.

Keywords: Melatonin; supplements; Intraocular pressure; coadjuvant treatments

Ocular hypertension is an anomalous physiological situation related to the appearance of ocular pathologies such as glaucoma. The relationship between ocular hypertension and glaucomatous pathology has been well known for many years. Even today, ocular hypertension is still considered the most important risk factor for the development of this disease [1].

Melatonin is an neurohormone that not only is produced by the pineal gland but is also synthesized and released by some ocular structures such as the retina, ciliary body or the lens [2]. Osborne described that melatonin is involved in the control of the aqueous humour dynamics[3]. In this sense, studies performed in animal models suggested that melatonin and its analogues can reduce IOP in normotensive and hypertensive eyes[4]. This control of IOP carried out by melatonin has invited to use of this compound and its analogues for the treatment of ocular hypertension and glaucoma[5]. There are some studies in humans that show the similar effect after melatonin intake [6-8].

A new nutritional supplement as coadjuvant treatment has been developed, based on melatonin to reduce the IOP and other components to improve the other three fundamental concerns of glaucomatous pathology: the elimination of free radicals, the protection and reinforcement of the nervous system and the increase in retinal blood supply [9, 10]. The objective of this study was to evaluate the possible acute and daily effect of a new nutritional supplement based on melatonin on the intraocular pressure (IOP) in normotensive subjects.

A short-term, prospective and randomized study has been performed. Sixty-seven normotensives subjects were recruited from the Optometry Clinic of the Faculty of Optics and optometry (University Complutense of Madrid, Spain). –Mean age was 42.64 ± 9.01 years (range 23 to 54 years), being 43 men and 24 women. Before

beginning the study, the risks and benefits of the treatment were explained, and informed consent was obtained from all subjects. Once included, each subject was examined by ophthalmologist in order to detect any eye disease not mentioned before. Participants were free to leave the study at any time. The study was conducted in compliance with good clinical practice guidelines, institutional review board regulations (IRB number approval: 18.444 O-P) and the tenets of the Declaration of Helsinki revised in 2013 [11].

A specific nutritional supplement for glaucoma was evaluated (Visaid Press; Avizor SA; Madrid, Spain). This supplement is based on melatonin (1 mg), due its antioxidant and hypotensor efficacy [10], and accompanied by vitamin B6 for its properties acting on the nervous system [12], anthocyanins, as blood flow promoter [13], Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA) and manganese. The supplements are administered as a gel pill. The manufacturer intake recommendation is one pill at night.

Subjects were randomly divided in two groups. The daily group (DG) (n=18) were instructed to take the supplement from 22.00 to 23.00, before going to sleep, during 3 consecutive days. IOP was measured at 10.00 and 11.00 am one day before, as baseline, and during the 3 days of experiment at the same time points. The acute group (AG) (n=49) was instructed to take the supplement at the morning only during one day. IOP was measured 1 hour and just before to take the supplement and one and two hours after. All measures in this group were taken one day ~~after~~ before without any supplement (control) and the day of experiment by the same experienced examiners (C.R and J.B.).

For IOP measurement, VX-130 platform (Luneau, Technologies, Chartres, France) was used. The VX130 is a multi-diagnostic platform that combines non-contact tonometer (air puff based) with Hartmann–Shack based autorefraction and Placido-disk and

Scheimpflug based corneal-topography. Three measurements were taken on each subject and the average of the readings was recorded as the final IOP. The mean of measures at 10.00 and 11.00 was considered as basal value for each day. During the measurement, subjects were asked to keep the eye open and fixate to the light stimulus of the device.

Data were analysed by statistical package SPSS version 22.0 for Windows (SPSS, Inc., Chicago, IL). The values presented are the means ± SD of the experiments performed. Parametric tests were used to compare the studied groups. Differences between baseline and after supplement intake in both groups were estimated by the Student - t test for related samples. One-way ANOVA for related samples were used to evaluate the trend of IOP in DG group. P <0.05 was considered statistically significant.

The DG group showed a significant decreasing in IOP after supplement intake in all days of experiment compared with baseline, being 14.9 ± 3.4 mmHg for baseline and 14.1 ± 2.7 mmHg, 13.6 ± 2.7 mmHg and 13.8 ± 2.9 mmHg after 24 hours, 48 hours and 72 hours of experiment, respectively (p value<0.001; Student t-test for related samples). Moreover, the IOP trend during the complete experiment was to decrease, being statistically significant (p value<0.05; One-way ANOVA for related) Figure 1.

Regarding AG group, IOP did not change during the control day, being 15.7 ± 2.4 mmHg before to supplement intake and 15.6 ± 2.5 mmHg and 15.6 ± 2.6 mmHg after two and three hours, respectively (p value > 0.05; Student t-test for related samples). However, a reduction of 1 mmHg was found 2 hours after supplement intake, from 15.7±2.5 mmHg to 14.7±2.5 mmHg, being statistically significant (p value < 0.05; Student t-test for related samples). After three hours, IOP followed being statistically inferior than baseline, with a mean value of 15.1±2.7 mmHg (p value < 0.05; Student t-test for related samples). Figure 2.

The nutritional supplement based on melatonin decreased IOP in normotensive subjects in both experiments performed. A single dose reduces IOP around 7% after two hours of its ingest and the consecutive intake for three days was able to reduce it around 9%.

The effect of melatonin or analogues over IOP has been previously described in humans by oral intake. Samples et al. found that oral melatonin administration (500 mg) reduced IOP in normal subject around 10% [8]. Pescosolido et al. found that agomelatine, a melatonin analogue, was able to reduce IOP around 30% after oral treatment in a group of primary open angle glaucoma patients. These patients were treated with 25 mg of agomelatine for psychiatric conditions [6]. Another study, published in 2009, has demonstrated IOP reduction when patients that underwent cataract surgery were treated with melatonin. These patients were treated with 10 mg of melatonin and IOP decreased significantly from 17.9 ± 0.9 to 14.2 ± 1.0 mmHg after premedication and to 13.8 ± 1.1 mmHg during surgery [7]. Both studies found an IOP reduction higher that the present trial probably due to the different concentration ingested and to the initial patient IOP.

The main limitation of the study is that there is no placebo group, but results are compared to the measures of the same patient at the same time points in the previous day. It should also be considered that ~~the use~~ measures are performed ~~of~~with non-contact tonometer based on air-puff. Some studies found that the non-contact tonometry gives higher results than Goldman tonometry, especially in adult subjects and hypertensive patients [14]. But, the repeatability of non-contact tonometry has been previously described in normotensive population [15]. It ~~is~~ should be important to perform the same study with a placebo group and Goldman or Perkins tonometry in

both normotensives and hypertensive patients to corroborate the outcomes of this study.

The nutritional supplement evaluated is composed for other components with interesting features for glaucoma as it has been mentioned in methods section and, taking account the outcomes found in the current study, it would be interesting to design and develop some long-term studies to evaluate the supplement effect in retina protection.

In conclusion, the nutritional supplement based on melatonin seems to decrease the IOP in normotensive subjects. These outcomes indicate that melatonin supplementation could be a coadjuvant treatment of glaucoma. Nevertheless, it is mandatory to confirm this role in glaucomatous patients compared with placebo group and with more studies about IOP changes after supplement intake.

Compliance with Ethical Standards:

Funding statement: The authors do not have any financial interest on the materials and instruments used in this study and this study was not funded by third-parties.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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FIGURES LEGENDS

FIGURE 1. Effect on IOP during the three days of experiment in daily group (DG). n=18. It is represented the mean and standard error of the mean (SEM). * p value<0.05; after intake vs before intake (PRE) (Student t-test for related samples).

FIGURE 2. Effect on IOP after two and three hours of a unique melatonin supplement intake in acute group (AG). n=49. It is represented the mean and standard error of the mean (SEM). * p value<0.05; after intake vs before intake (PRE) (Student t-test for related samples).

ABSTRACT

Purpose: To evaluate the effect of a new nutritional supplement based on melatonin on the intraocular pressure (IOP) in normotensive subjects.

Patients and Methods: A short-term and prospective study was designed. Sixty-seven normotensive subjects were recruited. Patients were divided in two groups. The daily group (DG) (n=18) were instructed to take the supplement from 22.00 to 23.00 (before sleeping) during 3 consecutive days. IOP was measured from 10.00 to 11.00 am the day before treatment and during the 3 days of experiment. The acute group (AG) (n=49) was instructed to take the supplement after the second measure (11.00) of the second day. IOP was measured 1 hour and just before the intake of the supplement and one and two hours after. All measures in this group were taken one day before without any supplement (control) and the day of experiment.

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Conclusion: The supplement based on melatonin was able to reduce the IOP in normotensives subjects after 2 hours of intake. Moreover, the daily intake showed a reduction of IOP during the three days of experiment.

Keywords: Melatonin; supplements; Intraocular pressure; coadjuvant treatments

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FIGURES LEGENDS

FIGURE 1. Effect on IOP during the three days of experiment in daily group (DG). n=18. It is represented the mean and standard error of the mean (SEM). * p value<0.05; after intake vs before intake (PRE) (Student t-test for related samples).

FIGURE 2. Effect on IOP after two and three hours of a unique melatonin supplement intake in acute group (AG). n=49. It is represented the mean and standard error of the mean (SEM). * p value<0.05; after intake vs before intake (PRE) (Student t-test for related samples).

FIGURE 1

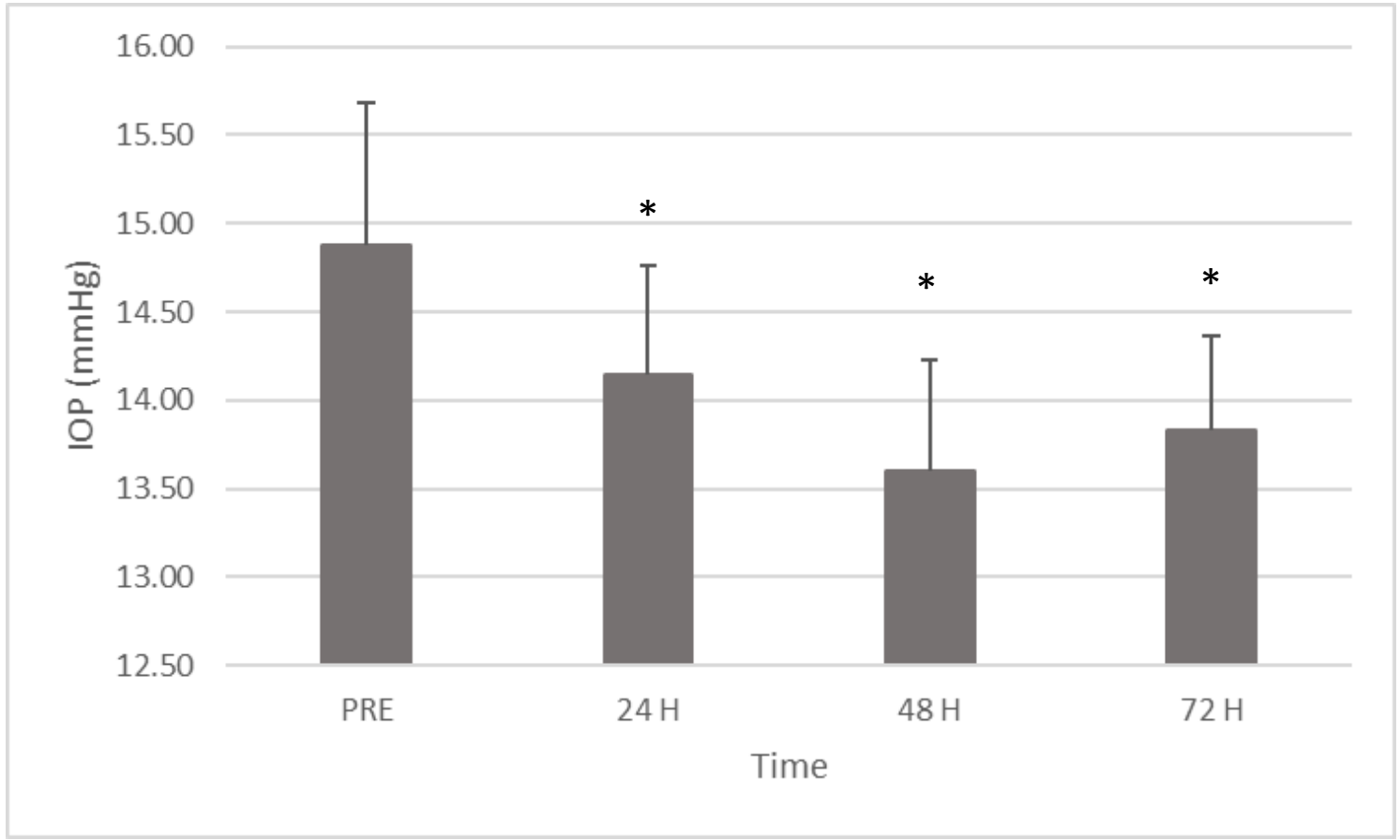

FIGURE 2